The characterization of glutathione S-transferases from rat olfactory epithelium

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The glutathione S-transferases (GSTs) of rat olfactory epithelium have been characterized with regard to substrate specificity and subunit composition and compared to those of the liver. The presence of cytosolic GST activity in rat olfactory epithelium was confirmed and, using 1-chloro-2,4-dinitrobenzene as substrate, was found to be approximately one-third that of the liver. Olfactory microsomal GST activity was greater than that of liver microsomes and could be activated by treatment with the sulphydryl agent *N*-ethylmaleimide. The subunit and isoenzyme profile of GSTs in the olfactory epithelium was investigated using a number of techniques. (1) Olfactory GSTs were characterized using a range of relatively subunit-specific substrates. Activities ranged from 40–90% of those found in liver. Most noticeable was the extremely low olfactory activity with the substrate specific for subunit 1. (2) Immunoblotting with antibodies against specific rat hepatic GSTs confirmed the presence of a number of subunits and the absence of subunit 1. (3) F.p.l.c. chromatofocusing and reverse-phase h.p.l.c. indicated that the cytosolic GST profile of olfactory epithelium is unique and is made up of subunits 2, 3, 4, 7, 8 and 11 with subunits 3 and 4 predominating. There is an absence of isoenzymes containing subunit 1.

INTRODUCTION

The recent discovery that the nasal epithelia of many species, including man, contain active drug-metabolizing enzyme systems raises fundamental questions regarding the physiological role and toxicological significance of these systems. It has been suggested that nasal drug metabolism may play an essential role in maintaining olfactory acuity (Dahl, 1986), but this still requires experimental verification. Inhalation is a major route of exposure to environmental chemicals and the toxicological significance of xenobiotic metabolism in the nasal epithelia is only beginning to be fully appreciated. *In situ* activation of chemicals within the nasal cavity can lead to the development of nasal lesions and tumours (Reed, 1993) and xenobiotic metabolism in nasal tissue may also play a role in protecting the lungs from toxic insult (Dahl, 1986). Finally, first-pass metabolism within the nasal cavity may be an important consideration in the design of drugs for intranasal administration.

In order to understand the implications of drug metabolism in the nasal cavity fully, the enzymes involved must be characterized. Much is known about nasal cytochromes *P*-450, which have been shown to have higher catalytic activity with many xenobiotic substrates than the hepatic haemoproteins (Reed et al., 1986). The nasal cytochrome *P*-450 isoenzyme profile has been investigated and a novel form, *P*-450IIG1, which is expressed only in the olfactory epithelium of the nasal cavity, has been identified (Nef et al., 1990). In contrast, there is a paucity of information regarding other nasal drug-metabolizing enzymes, in particular the glutathione S-transferases.

The glutathione S-transferases (GSTs; EC 2.5.1.18) are a group of enzymes that catalyse the conjugation of glutathione with a variety of electrophilic compounds, and both cytosolic and microsomal forms have been described (Mannervik and Danielson, 1988; Ketterer et al., 1988). The cytosolic GSTs are dimers formed by combinations of subunits, with M_r values in the range 24000–28000. The subunits are members of four multigene families known as Alpha, Mu, Pi and Theta. The Alpha family comprises subunits 1, 2, 8 and 10; the Mu family subunits 3, 4, 6, 9 and 11; subunit 7 is the only member of the Pi family and subunits 5 and 12 are members of the Theta family (Mannervik and Danielson, 1988; Ketterer et al., 1988; Kispert et al., 1989; Meyer et al., 1991). Within each family, subunits form either homodimers or heterodimers which exhibit broad and overlapping substrate specificities and immunological cross-reactivity with antisera. The microsomal GST is believed to be a trimer formed of three identical subunits, each with an M_r of 17200 (Morgenstern et al., 1985). Treatment with the sulphydryl agent *N*-ethylmaleimide activates the hepatic microsomal GST, resulting in a marked increase in catalytic activity (Morgenstern et al., 1984).

The aim of this study was to characterize nasal GSTs with respect to substrate specificity, immunological cross-reactivity and subunit composition.

MATERIALS AND METHODS

Chemicals

Reduced glutathione (GSH), 1-chloro-2,4-dinitrobenzene (CDNB), 1,2-epoxy-3-(*p*-nitrophenoxy)propane (EPNP), bromosulphophthalein (BSP), *trans*-4-phenyl-3-buten-2-one (*t*-PBO), cumene hydroperoxide (CuOOH), ethacrynic acid (EA), *N*-ethylmaleimide, acrylamide/bisacrylamide (40% solution), 5-bromo-4-chloro-3-indolyl phosphate (BCIP), Nitro Blue Tetrazolium (NBT), alkaline-phosphatase-conjugated anti-sheep

Abbreviations used: GST, glutathione S-transferase; CDNB, 1-chloro-2,4-dinitrobenzene; EPNP, 1,2-epoxy-3-(*p*-nitrophenoxy)propane; BSP, bromosulphophthalein; *t*-PBO, *trans*-4-phenyl-3-buten-2-one; CuOOH, cumene hydroperoxide; EA, ethacrynic acid; BCIP, 5-bromo-4-chloro-3-indolyl phosphate; NBT, Nitro Blue Tetrazolium; DCNB, 1,2-dichloro-3-nitrobenzene; AD, Δ^5-androstene-3,17-dione; TFA, trifluoroacetic acid; DCM, dichloromethane.

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IgG, S-hexylglutathione, epoxy-activated Sepharose 6B and GSH–agarose were all from Sigma Chemical Company. 1,2-Dichloro-3-nitrobenzene (DCNB) was from Aldrich Chemical Company. Δ^5-Androstene-3,17-dione (AD) was from Steraloids Inc., Wilton, NH, U.S.A. Antibodies against rat GSTs 1–1, 2–2, 3–3, 4–4, 7–7 and 8–8 were from Medlabs, Dublin, Ireland. Nitrocellulose, Pharmalyte 8–10.5 and Polybuffer 96 were purchased from Pharmacia. Trifluoroacetic acid (TFA) was from Applied Biosystems Ltd. Acetonitrile was from Romil Chemicals. All other chemicals were of the highest quality available commercially.

Preparation of tissue

Male Wistar rats (200–250 g) used in this study were allowed food and water *ad libitum*. Animals were killed by exposure to a rising concentration of CO_2. Liver and nasal cytosolic and microsomal fractions were prepared by differential ultracentrifugation of tissues homogenized in 0.25 M sucrose and 0.1 M Tris/HCl, pH 7.4, containing 1.15% KCl, respectively (Hadley and Dahl, 1982). All microsomal preparations were resuspended in 1.15% KCl and recentrifuged at 4 °C and 100000 *g* for 1 h to avoid contamination with cytosolic proteins.

Assays

GST activities with CDNB, DCNB, EPNP, EA, *t*-PBO and BSP as substrates were determined according to the method of Habig et al. (1974). The final concentrations of substrates were: CDNB and DCNB, 1 mM; EPNP, 0.5 mM; EA, 0.2 mM; *t*-PBO, 0.05 mM and BSP, 0.03 mM. The GSH concentration was 5 mM except in assays with EA and *t*-PBO (0.25 mM) and CDNB (1 mM). Activity with AD (68 μM) was determined by the method of Benson and Talalay (1976). GST conjugation of CuOOH (1.2 mM) was measured as described by Reddy et al. (1981). GST activity with dichloromethane (DCM) as substrate was measured by incubating cytosol at 37 °C in 0.2 M Tris/HCl buffer, pH 7.4, containing 5 mM $MgCl_2$ and 5 mM glutathione. The reaction was performed in sealed glass tubes and was initiated by the addition of 0.25% (v/v) DCM. Samples (1 ml) were removed at 10 min intervals and protein precipitated by the addition of 0.25 ml of ice-cold 20% (w/v) trichloroacetic acid. After centrifugation, 1 ml of supernatant was analysed for formaldehyde as described by Nash (1953).

Microsomal GST activity was assayed and activation by *N*-ethylmaleimide carried out according to the method of Morgenstern et al. (1980). To activate GST activity, 50 μl of microsomal protein was added to 450 μl of 1.11 mM *N*-ethylmaleimide in 0.1 M sodium phosphate buffer, pH 7.5, and incubated at room temperature for 30 s before assay. The GSH concentration was increased to 5 mM to saturate the microsomal activity.

Incubations contained 0.05–0.3 mg of either nasal or hepatic cytosolic protein except for the assay with AD and DCM as substrates when nasal cytosolic protein was increased to 0.6–0.7 mg. Protein concentrations were determined by the method of Lowry et al. (1951), using bovine serum albumin as standard.

SDS/PAGE and Western blotting

SDS/PAGE was carried out using a Bio-Rad Minielectrophoresis system with a 12% (w/v) resolving gel, as described by Laemmli (1970). Proteins were transferred to nitrocellulose by the method of Towbin et al. (1979). The blots were probed with antibodies against specific rat hepatic GSTs, 1–1, 2–2, 3–3, 4–4, 7–7 and 8–8, followed by alkaline-phosphatase-conjugated second antibody. BCIP was used in conjunction with NBT for the detection of the alkaline-phosphatase-conjugated complexes, as described by Blake et al. (1984).

Separation of GST isoenzymes by f.p.l.c.

F.p.l.c. was carried out as described by Ålin et al. (1985). Cytosolic protein was purified by affinity chromatography using *S*-hexylglutathione linked to epoxy-activated Sepharose 6B. The GST isoenzymes were separated by high-performance liquid chromatofocusing on a Pharmacia Mono P HR 5/20 column.

Separation of GST subunits by h.p.l.c.

Reverse-phase h.p.l.c. was carried out by the method of Ostlund Farrants et al. (1987), using a Dynamax C_{18} column (4.6 mm × 250 mm) (Rainin Instrument Co., Woburn, MA, U.S.A.). The solvents were 0.06% TFA in water (solvent A) and 0.04% TFA in acetonitrile (solvent B). The samples (1 ml), purified by affinity chromatography on GSH–agarose, were injected at 35% solvent B and a gradient was run from 35 to 65% (v/v) solvent B over 60 min, with a flow rate of 1 ml/min. Absorbances were measured at 214 nm and areas integrated using a Shimadzu C-R5A computing integrator. GST subunits were numbered as described previously by Mannervik and Danielson (1988), Ketterer et al. (1988) and Kispert et al. (1989).

Statistical analysis

The probability, *P*, of the significance of the difference between two sets of results was calculated by using the Student's *t*-test. Values of $P < 0.01$ were considered to indicate that two sets of results were statistically significantly different.

RESULTS

The cytosolic fractions of rat olfactory and respiratory epithelia exhibited significant GST activity with CDNB as the substrate. CDNB is conjugated at comparable rates by the majority of GST isoenzymes and is therefore used to determine the total GST activity. The specific activity of olfactory epithelium, 335 nmol/min per mg of protein, is approximately one-third that of the liver and is 2.6-fold higher than that of the respiratory epithelium (Table 1a). In addition to cytosolic GST activity, the olfactory epithelium was found to contain microsomal GST activity. Using CDNB as the substrate, GST activity in olfactory microsomes was 1.7-fold higher than that of hepatic microsomes (Table 1b), and could be increased 2–3-fold in both tissues by incubation with the sulphydryl agent *N*-ethylmaleimide.

Once the presence of GST activity in the cytosolic fraction of the olfactory epithelium had been established, the contribution to the total activity of different GST isoenzymes was investigated. A variety of substrates which are relatively specific for rat GST subunits were used and, as can be seen in Table 2, GST activity was detectable in olfactory epithelium with all the substrates except DCM.

Characteristic properties of Alpha class isoenzymes include the non-selenium-dependent glutathione peroxidase activity towards organic hydroperoxides (subunit 2), and isomerization of Δ^5-3-ketosteroids to Δ^4-3-ketosteroids (subunit 1). The activity with CuOOH was at least 2-fold greater in liver than olfactory

Table 1 GST activities of rat liver and nasal epithelia (a) cytosolic and (b) microsomal fractions

Activities were measured using CDNB as a substrate, as described in the Materials and methods section. Activation was performed by incubating microsomal protein in 1.11 mM *N*-ethylmaleimide (NEM) in 0.1 M sodium phosphate buffer, pH 7.5, at room temperature for 30 s before assay. All values are means ± S.D. of at least three separate determinations, each obtained with tissue pooled from three animals. * $P < 0.01$, when compared with corresponding value obtained with non-activated samples. † $P < 0.01$ when compared with corresponding value obtained with liver microsomes. N.D., not done.

Tissue	±NEM	Specific activity (nmol/min per mg of protein)
(a) Cytosolic		
Liver	N.D.	981.0 ± 45.9
Olfactory epithelium	N.D.	334.7 ± 95.1
Respiratory epithelium	N.D.	127.3 ± 6.9
(b) Microsomal		
Liver	−	131.5 ± 4.9
	+	441.7 ± 22.2*
Olfactory epithelium	−	220.7 ± 1.8†
	+	504.4 ± 30.0*

Table 2 GST activities of liver and olfactory cytosol with subunit-specific substrates

The assays were performed as described in the Materials and methods section. All values are means ± S.D. of at least three determinations, each obtained with tissue pooled from three animals.

Class	Subunit type	Substrate	Specific activity (nmol/min per mg of protein) Liver	Olfactory epithelium
General	General	CDNB	981.0 ± 45.9	334.7 ± 95.1
Alpha	1	AD	40.1 ± 7.1	0.9 ± 0.07
	2	CuOOH	52.5 ± 11.0	23.1 ± 11.3
	8	EA	10.6 ± 3.1	9.8 ± 1.9
Mu	3	DCNB	38.8 ± 9.0	25.2 ± 3.2
	3	BSP	6.3 ± 1.0	3.3 ± 1.0
	4	*t*-PBO	5.9 ± 0.6	4.5 ± 0.6
	4	EPNP	34.6 ± 5.1	21.7 ± 2.9
Theta	5	DCM	0.8 ± 0.08	< 0.01

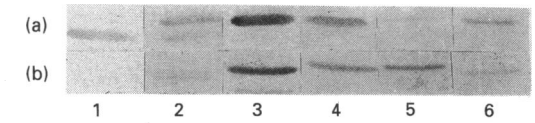

Figure 1 Western blot analysis of liver and olfactory cytosol

Experimental conditions were as described in the Materials and methods section. Aliquots containing (**a**) 30 μg of liver cytosol and (**b**) 30 μg of olfactory cytosol were subjected to SDS/PAGE and protein transferred to nitrocellulose, which was cut into strips and probed with different antibodies against specific rat hepatic GST isoenzymes. Lane 1, GST 1–1; lane 2, GST 2–2; lane 3, GST 3–3; lane 4, GST 4–4; lane 5, GST 7–7; lane 6, GST 8–8.

epithelium, whereas activity with AD was 44-fold greater in liver (40.1 nmol/min per mg of protein) than olfactory epithelium (0.9 nmol/min per mg of protein). This suggests a relative lack of isoenzymes containing subunit 1 in this tissue. Activity with EA, which is conjugated most actively by the 8–8 isoenzyme, was not significantly different in olfactory epithelium compared with the liver. The activity with substrates relatively specific for the Mu class isoenzymes DCNB, *t*-PBO and BSP were respectively 1.5-, 1.6- and 1.9-fold higher in liver than olfactory epithelium. Activity with DCM, a substrate for the 5–5 isoenzyme, was just detectable in liver, but below the limits of detection in the olfactory epithelium. However, using EPNP, which is more specific for the 5–5 isoenzyme, there was significant activity in both the liver and olfactory epithelium. The activity was 1.6-fold greater in the liver. Alpha and Mu class isoenzymes also contribute to EPNP conjugation; therefore the activity in olfactory epithelium may be due to subunits of the Mu class. GST isoenzymes exhibit broad and overlapping substrate specificities and therefore the results in Table 2 do not provide conclusive evidence as to the isoenzymes present in the olfactory epithelium or their relative proportions.

To confirm the presence of isoenzymes of the Alpha, Mu and Pi classes in olfactory epithelium, SDS/PAGE followed by Western blotting and immunodetection was performed. Liver cytosol cross-reacted with antibodies against rat hepatic 1–1, 2–2, 3–3, 4–4, 7–7 and 8–8 isoenzymes (Figure 1). Two bands were seen with the antibody against GST 2–2 due to cross-reactivity within the Alpha class. The upper band corresponds to subunit 2 and the lower band to subunit 1. The olfactory cytosol cross-reacted with the antibodies against GSTs 2–2, 3–3, 4–4, 7–7 and 8–8 but not against the 1–1 isoenzyme (lane 1). The apparent M_r of the bands in olfactory epithelium agreed with those in the liver and also with published values. The single band seen in lane 2 co-migrated with the upper band of liver cytosol and appears to be subunit 2.

To determine the relative proportions of each isoenzyme in the tissues, both f.p.l.c. and h.p.l.c. were employed. Isoenzymes from cytosol purified by affinity chromatography using *S*-hexylglutathione linked to epoxy-activated Sepharose 6B were separated by high-performance liquid chromatofocusing using an f.p.l.c. system. This system was used to obtain enzymically-active fractions.

The f.p.l.c. profiles of olfactory epithelium and liver were similar with six and seven peaks separated respectively over a pH range of 10.4–7.3 (Figure 2). Corresponding peaks in the liver and olfactory epithelium eluted at the same pH. Although there was less protein in all the olfactory epithelium peaks, peak A was obviously much smaller than the corresponding peak in liver. Since the liver profile was similar but not identical to published data (Ålin et al., 1985), the identity of the liver and corresponding olfactory epithelium peaks were determined by SDS/PAGE and immunoblotting, after the equivalent peaks from three chromatofocusing runs had been pooled and concentrated (results not shown). Although the liver peaks were identified, not all the peaks from olfactory epithelium could be identified in this way. This is probably due to the protein content in these peaks being below the limits of detection by immunoblotting, rather than to the presence of olfactory-specific GST isoenzymes.

Attempts to classify the peaks using GST activities with specific substrates were also unsuccessful because of the low protein concentration; therefore reverse-phase h.p.l.c. was used to determine the proportions of the different subunits in the tissue.

Figure 3 shows a typical example of an h.p.l.c. separation of GST subunits from rat olfactory epithelium (Figure 3a) and liver (Figure 3b). The liver cytosol resolved into seven peaks cor-

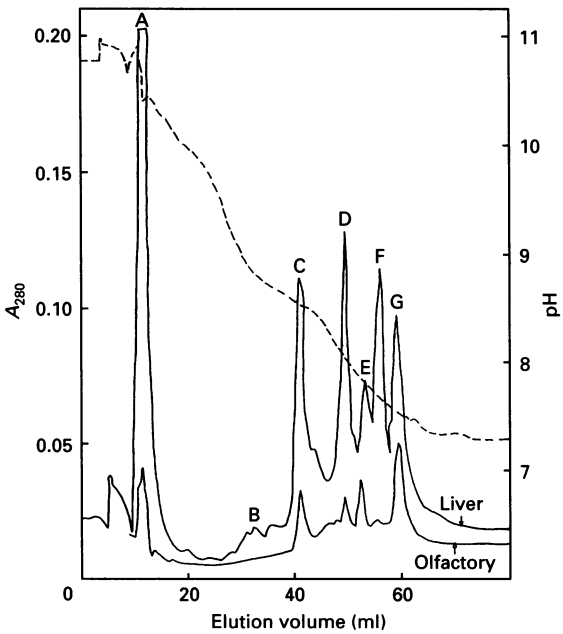

Figure 2 F.p.l.c. chromatofocusing of liver and olfactory cytosolic GSTs

GST isoenzymes from liver (2 mg) (**a**) and olfactory epithelium (1 mg) (**b**), eluted by *S*-hexylglutathione–Sepharose affinity chromatography were separated by f.p.l.c., as described in the Materials and methods section. The pH (– –) and A_{280} (——) were monitored.

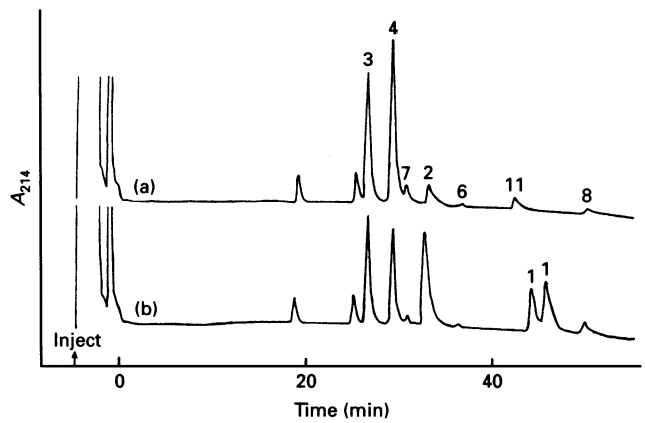

Figure 3 Separation of rat GST subunits of liver and olfactory cytosol by reverse-phase h.p.l.c.

Samples of approximately 50 μg of GSH–agarose-affinity-purified olfactory epithelium (**a**) and liver (**b**) GST subunits were separated by reverse-phase h.p.l.c., as described in the Materials and methods section. Numbers refer to GST subunits.

responding to subunits 3, 4, 7, 2, 6, 1 and 8, with retention times as previously reported (Ostlund Farrants et al., 1987). Subunit 1 separated into two peaks with the retention times of 1a and 1b (also known as Ya_1 and Ya_2) as reported by Ostlund Farrants et al. (1987). Only one peak was evident for subunit 2; this corresponds to the constitutively expressed Yc_1 subunit, previously known as Yc, rather than the ethoxyquin-inducible Yc_2 subunit which is not present in cytosol from adult rats and which has a marginally longer retention time (Hayes et al., 1991). The olfactory cytosol resolved into six peaks that had the same retention times as corresponding liver peaks (subunits 3, 4, 7, 2, 6 and 8). A seventh peak not seen in liver was also eluted. This

Table 3 Relative contribution of specific subunits isolated by h.p.l.c. to the total GST content of liver and olfactory epithelium

Reverse-phase h.p.l.c. was performed as described in the Materials and methods section and in the legend to Figure 3. Results are means ± S.D. of three separate determinations from three cytosolic preparations.

	Total area of peaks corresponding to GSTs* (%)	
Subunit	Liver	Olfactory epithelium
1a	11.2 ± 0.7	–
1b	15.1 ± 1.6	–
2	24.7 ± 0.4	8.8 ± 0.4
3	22.7 ± 1.6	33.2 ± 0.8
4	20.0 ± 1.8	44.7 ± 0.6
6	0.5 ± 0.2	0.1 ± 0.0
7	0.9 ± 0.3	5.3 ± 0.6
8	3.6 ± 0.2	3.2 ± 0.2
11	–	4.6 ± 0.2

* Total area of peaks that are GSTs: liver, 11.6×10^6 units; olfactory epithelium, 9.6×10^6 units.

peak eluted with a retention time of 42 min and is probably subunit 11 found in rat testis (Kispert et al., 1989). No peaks were eluted at the retention times corresponding to subunit 1. The two peaks seen before 25 min in both tissues were solvent effects. When expressed as a percentage of the total area corresponding to GST peaks (9.6×10^6 units for olfactory epithelium; 11.6×10^6 units for liver), 83% of the olfactory epithelium GSTs were Mu class subunits, compared with 43% in liver (Table 3). The Pi class (subunit 7) made up 5.3% of the olfactory epithelium GST area, 6-fold greater than the percentage in liver. Although the GST activity in the olfactory epithelium was found to be one-third that of the liver (Table 1), the total areas of GST peaks were not significantly different.

DISCUSSION

We have confirmed reports that the rat nasal epithelia contain significant GST activity, and have found cytosolic GST activity of the olfactory epithelium to be 2.6-fold higher than that of the respiratory epithelium (Table 1a), suggesting that GSTs are localized predominantly in olfactory tissue in the nasal cavity of rats. Studies on other drug metabolizing enzymes in the rat have shown that the nasal cytochromes P-450 (Dahl, 1985) and the carboxylesterases (Bogdanffy et al., 1987) are also preferentially located in olfactory epithelium. The cytosolic GST activity in olfactory epithelium using CDNB as the substrate (335 nmol/min per mg protein) is of a similar order of magnitude to values for other extrahepatic tissues, including kidney (Guthenberg et al., 1985), lung (Robertson et al., 1985) and small intestine (Tahir et al., 1988).

We have also demonstrated microsomal GST activity in olfactory epithelium. Morgenstern et al. (1982) found rates of CDNB conjugation in the microsomal fraction of rat liver to be significantly lower than in cytosol, and DeJong et al. (1988) have shown, using Northern blots, that the microsomal enzyme is most abundant in the liver, but is present in small amounts in many other tissues. The hepatic, but not the extrahepatic, enzymes have been shown to be activated several-fold by treatment with the sulphydryl agent *N*-ethylmaleimide (Morgenstern et al., 1980, 1984). Microsomal GST activity in

olfactory epithelium is comparable to cytosolic activity, higher than hepatic microsomal activity and can be activated by treatment with *N*-ethylmaleimide (Table 1). The function of microsomal GSTs has not yet been established but evidence suggests that they may inhibit lipid peroxidation (Morgenstern et al., 1990). If this is the case the microsomal GST of olfactory epithelium, which is in contact with an oxygen-rich environment, may offer protection to membrane lipids against oxidative damage, as well as detoxifying airborne xenobiotics.

We have analysed the isoenzyme and subunit profile of olfactory GSTs using a number of techniques. There was a marked absence of subunit 1 and a general paucity of Alpha class isoenzymes. Activity with AD was only detected at very low levels (Table 2) suggesting an absence of subunit 1; this was confirmed by the lack of cross-reactivity of olfactory cytosol with the antibody against rat hepatic GST 1–1 (Figure 1) and by f.p.l.c. and h.p.l.c. profiles (Figures 2 and 3, respectively). H.p.l.c. also demonstrated that subunits 2 and 8 are expressed in olfactory epithelium, but only at low levels (Figure 3). Subunit 1 is heterogeneous (Lai et al., 1984; Beale et al., 1982) and h.p.l.c. provides a means of separating it into at least two forms (Ostlund Farrants et al., 1987). These forms, also known as Ya_1 and Ya_2, have been shown, at the protein level, to be products of separate genes (Hayes et al., 1990). Neither form was detected in olfactory epithelium. Subunit 1 is also absent from rat lung (Robertson et al., 1985) and its absence may therefore be characteristic of respiratory tissues.

Recently Baron (1991), using immunofluorescence histochemical techniques, demonstrated significant amounts of GST 1–1 in the respiratory and olfactory epithelia of rats. This discrepancy with our results may be due to the high sensitivity of immunofluorescence techniques. However, Baron detected levels of 1–1 that were approximately 25% of those of subunits 3 and 4 combined, and we would expect to detect a subunit present at these levels by h.p.l.c. The antibody against hepatic isoenzyme 1–1 used in our study may be different in specificity from that used by Baron; alternatively, different strains of rats may have slightly different olfactory isoenzyme profiles.

Alpha class isoenzymes exhibit high activity with hydroperoxides derived from linoleate and arachidonate (Jensson et al., 1986; Ketterer et al., 1987) and with 4-hydroxyalk-2-enals produced during lipid peroxidation of biological membranes. The relative lack of Alpha class isoenzymes may therefore make the olfactory epithelium susceptible to damage by lipid peroxidation. The effect may be countered somewhat by the activity of the microsomal GST in olfactory epithelium. In addition, olfactory cytochromes *P*-450 have been shown to have high peroxidase activity using CuOOH as a substrate (Reed et al. 1988). Thus the lack of subunit 1 in olfactory epithelium may have been compensated for by other drug metabolizing enzymes.

All our data suggest that the majority of the olfactory GSTs are of the Mu class, with subunits 3 and 4 making up 78% of the total GSTs (Table 3). It is probable that subunit 11 is also present in the olfactory epithelium. This subunit is present at very low levels in rat liver but is a major component of other extrahepatic tissues including testis, epididymis and brain (Kispert et al., 1989). The significance of the high proportion of Mu class isoenzymes is as yet unknown.

Subunit 7 (Pi class) is present at higher levels in the olfactory epithelium than the liver (Table 3). This is seen in many extrahepatic tissues including the kidney, lung and small intestine, which is reported to be the richest source of this isoenzyme (Tahir et al., 1988). The reason for high extrahepatic levels of 7–7 is not known, but it has been suggested that they are a consequence of the rapid growth of epithelial cells. The olfactory epithelium, like the small intestine, is an epithelial tissue capable of rapid turnover.

We are unable to reach a conclusion regarding the presence of Theta class subunits 5 and 12 in olfactory epithelium. The Theta class isoenzymes do not bind to glutathione or *S*-hexylglutathione affinity matrices and are therefore not separated by the techniques used in this study. We found no activity with DCM in the olfactory epithelium, but the method for assay of DCM conjugation is relatively insensitive. Using immunofluorescence histochemical techniques Baron (1991) found significant levels of isoenzyme 5–5 in the nasal mucosa of rats. This point requires further clarification.

In summary, we have demonstrated the presence of GST activity in both the cytosolic and microsomal fractions of olfactory epithelium. The cytosolic profile is unique, and contains isoenzymes made up of subunits 2, 3, 4, 7, 8 and possibly also 11, with subunits 3 and 4 predominating, and an absence of the isoenzymes containing the subunit 1. Characterization of olfactory cytochromes *P*-450 has identified novel forms which are not found in the liver (Ding and Coon, 1988; Nef et al., 1990). We have no evidence for olfactory-specific GST isoenzymes.

Since CDNB conjugation in olfactory epithelium is one-third that in liver, it is reasonable to assume that the amount of GST protein in this tissue is considerably less than that in liver. This does not appear to be the case when considering the results of the h.p.l.c. analyses (Table 3). Liver and olfactory samples containing equivalent amounts of cytosolic protein were affinity purified and the same volume of purified GSTs injected onto the h.p.l.c. column. The total area of peaks (measured at 214 nm) corresponding to GSTs in both tissues was almost the same, from which we conclude that the GSTs constitute approximately the same percentage of cytosolic protein in each tissue. Experiments are currently underway to examine this discrepancy.

The physiological role of nasal drug-metabolizing enzymes such as the GSTs is not known, but they may play a role in protecting the brain from airborne xenobiotics. The olfactory neurons are in contact with the external environment and synapse directly in the brain, and it has been shown that xenobiotics applied to the olfactory epithelium or inhaled may undergo retrograde axonal transport and enter the brain, thus bypassing the blood–brain barrier (Shipley, 1985; Ghantous et al., 1990). The nasal drug metabolizing enzymes may therefore provide protection for the brain by detoxifying inhaled xenobiotics and limiting their access to the nervous system. Alternatively, they may activate innocuous compounds to toxic, carcinogenic or mutagenic species which are then transported to the brain. This line of study is now being investigated.

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